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SITE-SPECIFIC DNA DAMAGE INDUCED BY SULFITE IN THE PRESENCE OF COBALT(II) ION

ROLE OF SULFATE RADICAL

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Abstract—The reactivities of sulfite (SO_3^{2-}) with DNA in the presence of metal ions were investigated by a DNA sequencing technique using ^{32}P-labeled DNA fragments obtained from human c-Ha-*ras*-1 protooncogene. Sulfite caused DNA damage in the presence of Co^{2+}, Cu^{2+} and Mn^{2+}, although sulfite alone or metal ion alone did not. The order of inducing effect on sulfite-dependent DNA damage ($Co^{2+} \gg Cu^{2+} > Mn^{2+} > Fe^{3+}$) was consistent with that of accelerating effect on the initial oxygen consumption rate of sulfite autoxidation. The DNA damage induced by sulfite plus Co^{2+} was inhibited by 3,5-dibromo-4-nitrobenzenesulfonate, primary and secondary alchols, whereas it was not inhibited by SOD, catalase and *tert*-butyl alcohol. Incubation of DNA with sulfite plus Co^{2+} followed by the piperidine treatment led to the predominant cleavage at the positions of guanine especially located 5′ to guanine. Sulfite plus Cu^{2+} gave a DNA cleavage pattern different from that induced by sulfite plus Co^{2+}. The photolysis of peroxydisulfate ($S_2O_8^{2-}$), which is known to produce $SO_4^{\cdot-}$ radicals, gave a DNA cleavage pattern similar to that induced by sulfite plus Co^{2+}. ESR studies using spin-trapping reagent revealed the production of spin adduct possibly of $SO_3^{\cdot-}$ radical in a solution of sulfite plus Cu^{2+}, whereas much less spin adduct was produced by sulfite plus Co^{2+}. The results suggest that sulfite is rapidly autoxidized in the presence of Co^{2+} to produce $SO_4^{\cdot-}$ radical causing site-specific DNA damage.

Sulfite itself is used as a preservative in foods and drugs, and is formed in the lung by the hydration of sulfur dioxide, a major air pollutant. An epidemiological study has revealed that sulfur dioxide is associated with mortality from malignancies of the respiratory tract of males [1]. Although sulfite has not been shown to be a carcinogen in animal experiments, there is a possibility that it is a cocarcinogen or promoter [2–4]. Kuschner [2] reported that sulfur dioxide was cocarcinogenic for development of benzo[*a*]pyrene induced pulmonary carcinoma, which has been confirmed [3]. Takahashi *et al.* [4] also reported that metabisulfite exerted promoting activity in gastric carcinogenesis initiated by *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

Sulfite has been shown to induce neoplastic transformation of Syrian hamster cells [5], and sister chromatid exchange in Chinese hamster ovary cells [6]. Sulfite was originally found to be a base specific mutagen. At high concentration (1 M) and at pH 5, sulfite catalyzed deamination of cytosine to uracil in isolated DNA [7]. At lower concentrations, sulfite caused DNA damage by sulfite-generated free radicals [8]. Hayatsu and Miller showed by alkaline sucrose density gradient centrifugation that cleavage of DNA was induced by oxygen-dependent reaction of sulfite in the presence of Mn^{2+} [9]. However, the mechanism of DNA damage induced by low concentrations of sulfite remains to be clarified.

We have investigated (1) the reactivity of sulfite with ^{32}P-labeled DNA fragments obtained from human c-Ha-*ras*-1 protooncogene in phosphate buffer containing metal ions, and (2) the reaction mechanism by ESR spin-trapping techniques. The results suggest that sulfite is autoxidized in the presence of Co^{2+} ion to form $SO_4^{\cdot-}$ radical resulting in site-specific DNA cleavage.

MATERIALS AND METHODS

Materials. Restriction enzymes (*Bst*EII, *Ava*I, *Xba*I) and T_4 polynucleotide kinase were obtained from Toyobo Co. (Osaka, Japan). Calf intestine phosphatase was obtained from Boehringer Manheim GmbH (Mannheim, F.R.G.). [γ-^{32}P]-ATP (6000 Ci/mmol) was supplied by New England Nuclear (Boston, MA). DTPA† was obtained from Dojin Chemicals Co. (Kumamoto, Japan). Sodium sulfite, alcohols, $CoCl_2$, $CuCl_2$, $MnCl_2$, $FeCl_3$, $NiCl_2$, $ZnCl_2$, $CdCl_2$ and potassium peroxydisulfate were from Nakarai Chemicals Co. (Kyoto, Japan). Agarose was from Takara Shuzo Co. (Kyoto, Japan). DMPO and dimethyl sulfate were from Aldrich Chemical Co. (Milwaukee, WI). Acrylamide, bisacrylamide, and piperidine were from Wako Chemicals Co. (Osaka, Japan). Methional was from Sigma Chemical Co. (St Louis, MO). A stock solution of 0.1 M sodium sulfite was made up fresh when required.

Preparation of ^{32}P *5′-end-labeled DNA fragments.* Plasmid pbcNI which carries a 6.6-kilobase *Bam*HI chromosomal DNA segment containing human c-

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† Abbreviations used: DTPA, diethylenetriaminepentaacetic acid; DMPO, 5,5-dimethylpyrroline-*N*-oxide; DMPO-$SO_3^{\cdot-}$, $SO_3^{\cdot-}$ radical adduct of 5,5-dimethylpyrroline-*N*-oxide; DBNBS, sodium 3,5-dibromo-4-nitrosobenzenesulfonate; SOD, superoxide dismutase.

Ha-*ras*-1 protooncogene was purchased from American Type Culture Collection [10]. The plasmid was digested with *Bst*EII and *Ava*I, and the resulting DNA fragments were fractionated by electrophoresis on 2% agarose gels. A ^{32}P 5′-end-labeled 602-base pair *Ava*I fragment (*Ava*I*1645–*Ava*I*2246) from a 1.5-kilobase *Bst*EII fragment of pbcNI was obtained by dephosphorylating with calf intestine phosphatase and rephosphorylating with [γ-^{32}P]-ATP and T_4 polynucleotide kinase. The ^{32}P 5′-end-labeled 602-base pair *Ava*I fragment was further digested with *Xba*I to obtain a singly labeled 341-base pair fragment (*Xba*I 1906–*Ava*I*2246) and a 261-base pair fragment (*Ava*I*1645–*Xba*I 1905). The asterisk indicates ^{32}P-labeling and nucleotide numbering starts with *Bam*HI site [11].

Detection of DNA damage induced by sulfite. The standard reaction mixture in a microtube (1.5 ml Eppendorf) contained 1 mM sodium sulfite, 20 μM metal ion and [^{32}P]DNA fragment in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA. After the incubation for the indicated period at 37°, the DNA fragments were precipitated with cold ethanol and dried in a vacuum desiccator, followed by heating at 90° for 20 min in 1 M piperidine when necessary. The DNA fragments were electrophoresed using a 12 cm × 16 cm slab gel, and the autoradiograms were obtained by exposing X-ray film to the gels at −20° overnight as previously described [12–14].

The preferred cleavage sites by sulfite plus metal ions were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam-Gilbert procedure [15] using a DNA sequencing system (LKB2010 Macrophor). A laser densitometer (LKB 2222 UltroScan XL) with a linear response range with 4 O.D. was used for the measurement of the relative amounts of oligonucleotides from treated DNA fragments. In the photolysis experiment, the reaction mixture containing 3 mM potassium peroxydisulfate was illuminated for 2 min with a UV lamp (10 W, Manasulu Co., Tokyo, Japan) emitting 253.6 nm light placed at the distance of 5.5 cm.

Measurements of oxygen consumption during the autoxidation of sulfite. Oxygen consumption by the autoxidation of sulfite was measured in a thermostated (25°) water-jacketed glass vessel, fitted with a Clark electrode (Gilson).

ESR spectra measurements. DMPO was used as the spin-trapping reagent. Immediately after 72 mM DMPO was added in 20 mM sodium phosphate buffer at pH 7.9 containing 5 mM sulfite, 0.1 mM metal ion and 5 μM DTPA, aliquots of the solution were taken in a calibrated capillary, and ESR spectra were measured at room temperature using a JES-FE-3XG spectrometer with 100 KHz field modulation according to the previously described method [12]. Spectra were recorded with a microwave power of 1 mW and a modulation amplitude of 0.5 G. The magnetic fields were calculated by the splitting of Mn^{2+} in MgO (ΔH_{3-4} = 86.9 G).

RESULTS

Damage of ^{32}P labeled DNA fragments by sulfite plus metal ions

We examined whether sulfite causes DNA damage in the presence of metal ions using ^{32}P 5′-end-labeled DNA fragments. The extent of DNA damage was estimated by gel electrophoretic analysis. Figure 1 shows the effect of metal ions on sulfite-dependent DNA damage. The upper band and lower band in the control show double-stranded and single-stranded forms of DNA fragment, respectively. No oligonucleotides were observed with sulfite alone (Fig. 1; lane 2), showing that sulfite itself is not a DNA-damaging agent. Co^{2+}, Cu^{2+} and Mn^{2+} induced DNA damage in the presence of sulfite (Fig. 1; lanes 3, 4 and 5), whereas Fe^{3+}, Ni^{2+}, Zn^{2+} and Cd^{2+} showed little or no effect under the present conditions. Metal ions alone induced little or no DNA damage in the absence of sulfite. The order of ability to induce DNA damage with sulfite was $Co^{2+} \gg Cu^{2+} > Mn^{2+}$. Even without piperidine treatment, oligonucleotides were formed by sulfite plus Co^{2+}, Cu^{2+} or Mn^{2+}, suggesting the breakages of deoxyribose-phosphate backbone. The amounts of oligonucleotides increased with piperidine treatment. Since altered base is readily removed from its sugar by the piperidine treatment [15], it is considered that the base alterations and/or liberations were induced by sulfite plus Co^{2+}, Cu^{2+} or Mn^{2+}.

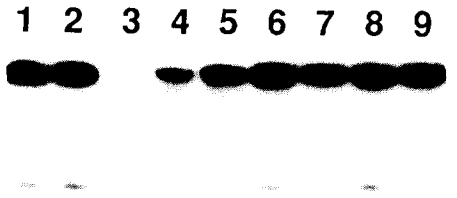

Fig. 1. Autoradiogram of ^{32}P-labeled DNA fragments incubated with sulfite in the presence of metal ions. Reaction mixture contained the ^{32}P-labeled 261-base pair fragment (*Ava*I*1645-*Xba*I1905) and 5 μM DTPA in 200 μl of 10 mM sodium phosphate buffer at pH 7.9. Where indicated, 2 mM sulfite and/or 20 μM metal ion was added. Lane 1, none; lane 2, SO_3^{2-}; lane 3, SO_3^{2-} + Co^{2+}; lane 4, SO_3^{2-} + Cu^{2+}; lane 5, SO_3^{2-} + Mn^{2+}; lane 6, SO_3^{2-} + Fe^{3+}; lane 7, SO_3^{2-} + Ni^{2+}; lane 8, SO_3^{2-} + Zn^{2+}; lane 9, SO_3^{2-} + Cd^{2+}. After the incubation for 60 min at 37°, the mixture was precipitated with cold ethanol, heated at 90° for 20 min in 1 M piperidine, and the DNA fragments were electrophoresed on an 8% polyacrylamide/8 M urea gel and the autoradiogram was obtained by exposing X-ray film to the gel.

To investigate whether superoxide and hydrogen peroxide are involved in the reaction of DNA with sulfite plus Co^{2+}, the effects of SOD and catalase were examined. DNA damage induced by sulfite plus

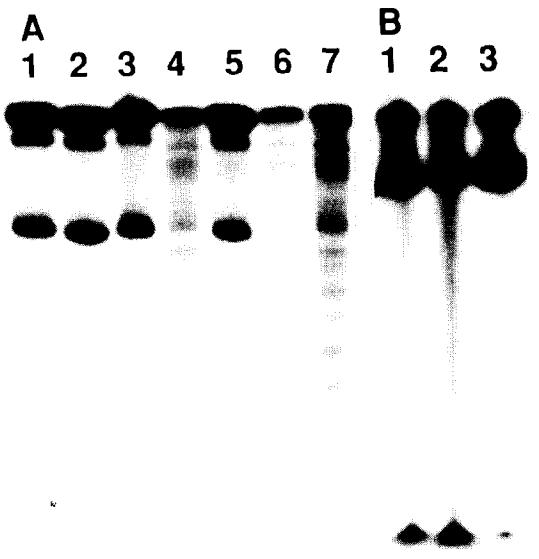

Fig. 2. Effects of SOD, catalase and DBNBS on DNA damage induced by sulfite in the presence of Co^{2+} or Mn^{2+}. (A) The ^{32}P 5′-end-labeled 341-base pair fragment (*Xba*I1906–*Ava*I*2246) in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA was incubated for 10 min at 37° with 1 mM SO_3^{2-} (lane 2); 20 μM Co^{2+} (lane 3); SO_3^{2-} + Co^{2+} (lane 4); SO_3^{2-} + Co^{2+} + 0.1 M DBNBS (lane 5); SO_3^{2-} + Co^{2+} + 30 units of catalase (lane 6); SO_3^{2-} + Co^{2+} + 30 units of SOD (lane 7). Lane 1 shows the electrophoresis pattern of the untreated ^{32}P-DNA fragment. (B) The fragment in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA was incubated for 120 min at 37° with 6 mM SO_3^{2-} + 20 μM Mn^{2+} (lane 1); SO_3^{2-} + Mn^{2+} + 30 units of catalase (lane 2); SO_3^{2-} + Mn^{2+} + 30 units of SOD (lane 3). After the piperidine treatment the DNA fragments were analysed by the method described in Fig. 1.

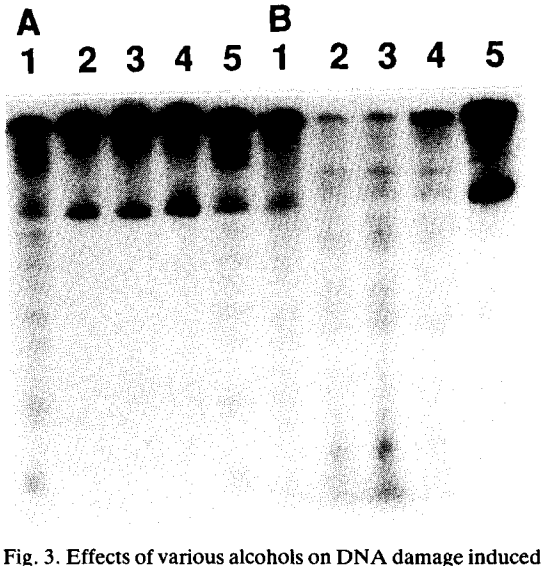

Fig. 3. Effects of various alcohols on DNA damage induced by sulfite in the presence of Co^{2+} or Cu^{2+}. (A) The ^{32}P 5′-end-labeled 341-base pair fragment (*Xba*I1906–*Ava*I*2246) in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA was incubated for 10 min at 37° with 1 mM SO_3^{2-} + 20 μM Co^{2+} (lane 1); SO_3^{2-} + Co^{2+} + 2% (v/v) ethanol (lane 2); SO_3^{2-} + Co^{2+} + 2% isopropyl alcohol (lane 3); SO_3^{2-} + Co^{2+} + 2% *n*-butyl alcohol (lane 4); SO_3^{2-} + Co^{2+} + 2% *tert*-butyl alcohol (lane 5). (B) The fragment in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA was incubated for 60 min at 37° with 1 mM SO_3^{2-} + 20 μM Cu^{2+} (lane 1); SO_3^{2-} + Cu^{2+} + 2% (v/v) ethanol (lane 2); SO_3^{2-} + Cu^{2+} + 2% isopropyl alcohol (lane 3); SO_3^{2-} + Cu^{2+} + 2% *n*-butyl alcohol (lane 4); SO_3^{2-} + Cu^{2+} + 2% *tert*-butyl alcohol (lane 5). After the piperidine treatment, the DNA fragments were analysed by the method described in Fig. 1.

Co^{2+} (Fig. 2A; lanes 6 and 7) was not affected by catalase and SOD, whereas DNA damage induced by sulfite plus Mn^{2+} was inhibited by SOD (Fig. 2B; lane 3). DBNBS, which is reported to react with SO_3^- radical [16], inhibited DNA damage (Fig. 2A; lane 5).

Effects of various alcohols on DNA damage induced by sulfite in the presence of Co^{2+} *or* Cu^{2+}

Figure 3A shows the effects of alcohols on DNA damage induced by sulfite plus Co^{2+}. Ethanol, isopropyl alcohol, and *n*-butyl alcohol inhibited DNA damage induced by sulfite plus Co^{2+} (Fig. 3A; lanes 2, 3 and 4). Methanol, *n*-propyl alcohol, *sec*-butyl alcohol and isoamyl alcohol also showed the inhibitory effect (data not shown). *tert*-Butyl alcohol did not inhibit DNA damage (Fig. 3A; lane 5). The effects of alcohols on DNA damage induced by sulfite plus Cu^{2+} was completely different from that induced by sulfite plus Co^{2+}. Ethanol, isopropyl alcohol, and *n*-butyl alcohol accelerated DNA damage induced by sulfite plus Cu^{2+} (Fig. 3B; lanes 2, 3 and 4), whereas *tert*-butyl alcohol did not accelerate it (Fig. 3B; lane 5). The effects of alcohols on DNA damage induced by the photolysis of peroxydisulfate were similar to those in the case of sulfite plus Co^{2+} (data not shown).

Specificity of cleavage of ^{32}P *5′-end-labeled DNA fragments treated with sulfite plus metal ions*

To estimate the site specificity of DNA cleavage, ^{32}P 5′-end-labeled DNA fragments treated with sulfite plus Co^{2+} or Cu^{2+} were electrophoresed and the autoradiogram was scanned with a laser densitometer (Fig. 4). The cleavage sites were determined by utilizing the Maxam-Gilbert procedure [15]. Without piperidine treatment, DNA cleavage by sulfite plus Co^{2+} occurred at the positions of every nucleotide without marked site-specificity (data not shown). With piperidine treatment, the cleavages at the positions of guanine, especially located 5′ to guanine, increased predominantly (Fig. 4B). Incubation of DNA with sulfite plus Cu^{2+} followed by the piperidine treatment seems to cause the relatively strong cleavages at the positions of guanine located 5′ to guanine and at the positions of pyrimidine located 5′ to the sequence G–G, although the site specificity was not so clear as that induced by sulfite plus Co^{2+} (Fig. 4C). The photolysis of peroxydisulfate gave a pattern similar to that given by sulfite plus Co^{2+} (Fig. 4A). Little or no DNA cleavage

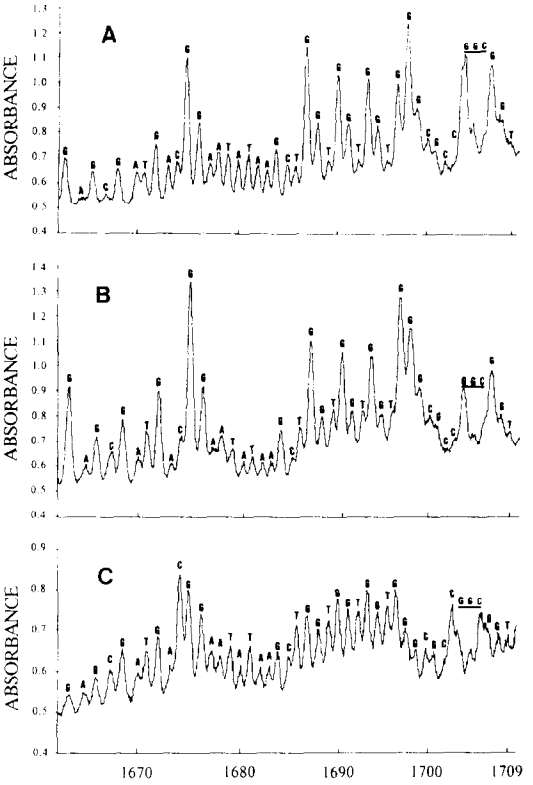

Fig. 4. Alkali-labile sites in ^{32}P-labeled DNA fragments treated with peroxydisulfate plus light, sulfite plus Co^{2+}, or sulfite plus Cu^{2+}. (A) The ^{32}P 5′-end-labeled 261-base pair fragment (*AvaI**1645–*XbaI*1905) in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA was illuminated in the presence of 3 mM $S_2O_8^{2-}$ as described in Materials and Methods. (B) The fragment in 200 μl of 10 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA was incubated with 1 mM sulfite plus 20 μM Co^{2+} for 10 min at 37°. (C) The fragment was incubated with 2 mM sulfite plus 20 μM Cu^{2+} for 120 min at 37°. After the piperidine treatment, DNA fragments were electrophoresed on an 8% polyacrylamide/8 M urea gel and the autoradiogram was obtained by exposing X-ray film to the gel. The relative amounts of oligonucleotides produced were measured by a laser densitometer (LKB 2222 UltroScan XL). The alkali-labile sites of the treated DNA were determined by direct comparison with the same DNA fragment after undergoing DNA sequence reaction according to the Maxam–Gilbert procedure [15]. The horizontal axis: the nucleotide number of human c-Ha-*ras*-1 protooncogene starting with *Bam*HI site [11]. *A*, *G*, *T*, and *C*, deoxyadenylate, deoxyguanylate, deoxythymidylate and deoxycytidylate of DNA, respectively. Underscoring: the 12th codon of human c-Ha-*ras*-1 protooncogene.

was induced by the illumination in the absence of peroxydisulfate or by the addition of peroxydisulfate in the dark.

In the case of Co^{2+}, alteration of the first guanine in the 12th codon of c-Ha-*ras*-1 protooncogene seems to be comparatively strong, whereas in the case of Cu^{2+}, alteration of the cytosine in the codon seems to be comparatively strong under the present conditions.

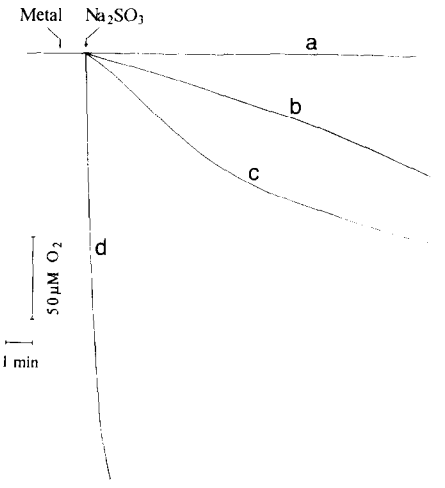

Fig. 5. Time course of O_2 consumption during the autoxidation of sulfite in the presence of metal ions. O_2 consumption was measured in a thermostated (25°) water-jacketed glass vessel, fitted for oxygen measurement with a Clark electrode (Gilson). Metal chloride (20 μM) was added to 1.7 ml of 20 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA. After 1 min, sodium sulfite (1 mM) was added; (a) no metal; (b) Mn^{2+}; (c) Cu^{2+}; (d) Co^{2+}.

Effects of metal ions on autoxidation of sulfite

Figure 5 shows the effect of metal ions on the O_2 consumption during the autoxidation of sulfite. Co^{2+} accelerated strongly the O_2 consumption during autoxidation of sulfite. Cu^{2+} accelerated the initial O_2 consumption. Mn^{2+} autocatalytically accelerated the O_2 consumption. Fe^{3+} showed little effect (data now shown). The order of catalytic ability in sulfite autoxidation was as follows: $Co^{2+} \gg Cu^{2+} > Mn^{2+} > Fe^{3+}$. SOD inhibited the acceleration of Mn^{2+}-dependent O_2 consumption but did not inhibit the acceleration of Co^{2+}-dependent or Cu^{2+}-dependent O_2 consumption (data not shown).

Sulfite radical production during the autoxidation of sulfite in the presence of metal ions

The spin-trapping method was used to detect free radicals produced during the autoxidation of sulfite in the presence of metal ions. Figure 6A shows the spectrum of spin adduct of DMPO obtained when DMPO was added into the solution containing sulfite and Cu^{2+}. The six lines can be interpreted in terms of hyperfine splitting constants (a_N = 14.7 G, a_H = 16.0 G) due to both the nitroxide nitrogen atom and the β-hydrogen. The hyperfine splitting constants are in good agreement with those reported for DMPO–SO_3^- [17, 18]. No spectrum of the DMPO spin adduct was observed with either sulfite or Cu^{2+} alone (Fig. 6B and C). In the case of Co^{2+}, the DMPO spin adduct was formed only less than 1/100 of the Cu^{2+}-dependent spin adduct. Mn^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+} and Cd^{2+} showed little effect (data not shown).

DISCUSSION

The present results show that although sulfite itself

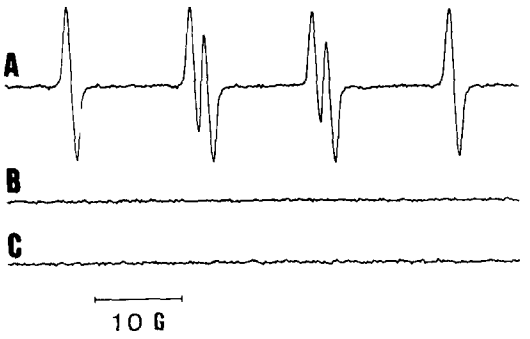

Fig. 6. ESR spectra of the sulfite radical spin adduct of DMPO produced during the autoxidation of sulfite in the presence of metals. Spectrum A: sample (200 μl) contained 5 mM sulfite plus 0.1 mM Cu^{2+} in 20 mM sodium phosphate buffer at pH 7.9 containing 5 μM DTPA. Spectrum B: sample (200 μl) contained 0.1 mM Cu^{2+} in the buffer. Spectrum C: sample (200 μl) contained 5 mM sulfite in the buffer. After 72 mM DMPO was added, aliquots of the solutions were taken in calibrated capillaries, and ESR spectra were measured at room temperature as described in Materials and Methods.

did not cause DNA damage, the addition of Co^{2+}, Cu^{2+} and Mn^{2+} induced DNA damage. Fe^{3+}, Ni^{2+}, Zn^{2+} and Cd^{2+} did not show any effect under the present conditions. Order of inducing effects on sulfite-dependent DNA damage was $Co^{2+} \gg Cu^{2+} > Mn^{2+}$. The order was consistent with that of catalytic activity of sulfite autoxidation. The DNA damage seems to be closely related to sulfite autoxidation. The autoxidation of sulfite in the presence of Co^{2+} and Cu^{2+} was not inhibited by SOD, whereas Mn^{2+}-dependent autoxidation was inhibited by SOD. The mechanism for Mn^{2+}-catalysed autoxidation of sulfite seems to be different from that for the Co^{2+} or Cu^{2+}-catalysed autoxidation. As Yang pointed out [19], superoxide was shown to be involved in the chain reaction of Mn^{2+}-catalysed autoxidation of sulfite. However, superoxide did not play an important role in Co^{2+} or Cu^{2+}-catalysed autoxidation of sulfite.

SOD and catalase did not inhibit DNA cleavage induced by sulfite plus Co^{2+}, whereas DBNBS inhibited it. Similar results were obtained in the case of sulfite plus Cu^{2+}. These results suggest that the SO_3^- radical or the active species derived from the SO_3^- radical and not oxygen free radicals participate in DNA damage induced by sulfite plus Co^{2+} or Cu^{2+}. On the other hand, SOD inhibited DNA cleavage induced by sulfite plus Mn^{2+}, suggesting oxygen radical participation in Mn^{2+}-dependent DNA damage.

The difference in the effects of alcohols on DNA damage led us to the idea that active species causing DNA damage in the case of sulfite plus Co^{2+} is different from that in the case of sulfite plus Cu^{2+}. The effects of alcohols on DNA damage induced by the photolysis of peroxydisulfate were similar to those in the case of sulfite plus Co^{2+}. The photolysis of peroxydisulfate is known to result in formation of two SO_4^- radicals [20]. The SO_4^- radical readily reacts with alcohols, whereas the SO_3^- radical and the SO_5^- radical are relatively inert to alcohols [21]. The reaction of the SO_4^- radical with *tert*-butyl alcohol is reported to be considerably slower than that with secondary or primary alcohols [21, 22]. It is inferred from the nature of the inhibition by alcohols that the SO_4^- radical participates in the sulfite plus Co^{2+}-induced DNA damage, and participation of the SO_4^- radical in the sulfite plus Cu^{2+}-induced DNA damage is small, if any. It is known that the initiating one-electron oxidation of sulfite yields an SO_3^- radical, which reacts rapidly with O_2 to produce an SO_5^- radical [23, 24]. The SO_4^- radical has not been reported to be an intermediate in metal-catalysed sulfite autoxidation. It is noteworthy that the SO_4^- radical is an active intermediate in the sulfite plus Co^{2+}-induced DNA damage.

ESR experiments demonstrated that the addition of Cu^{2+} to sulfite solution produced DMPO–SO_3^-. It suggests that Cu^{2+}-catalysed autoxidation of sulfite proceeds via the SO_3^- radical as an intermediate species. On the other hand, Co^{2+} showed much less production of the radical adduct than Cu^{2+}. The results suggest that the SO_3^- radical plays an important role in DNA damage induced by sulfite plus Cu^{2+}, whereas the SO_3^- radical participates little in DNA damage induced by sulfite plus Co^{2+}.

The DNA sequencing experiments on DNA fragments treated with sulfite plus Co^{2+} revealed that every deoxyribose-phosphate ester was almost equally decomposed without the piperidine treatment. The piperidine treatment increased the cleavages especially at guanine residues, indicating the alkali-labile lesions at guanine bases. The site specificity of DNA cleavage induced by sulfite plus Co^{2+} was essentially identical with that induced by the photolysis of peroxydisulfate. This supports the idea that SO_4^- radical is produced from sulfite plus Co^{2+} and specifically causes guanine alteration. Our previous paper suggested that hydroxyl radicals caused cleavages at every nucleotide with a little stronger cleavage at the positions of every guanine and thymine [12]. The difference of the reactivities with thymine between the SO_4^- radical and hydroxyl radical is explained by the character of the hydroxyl radical which has a stronger tendency toward the addition to double bonds than the SO_4^- radical [25]. Nakayama *et al.* reported that the energy level of the highest occupied molecular orbital of guanine is highest among the nucleic acid bases and accordingly guanine is oxidized most easily [26]. The predominant guanine alteration in Co^{2+}-sulfite dependent DNA damage may be due to the fact that the SO_4^- radical is a very effective electron-transfer oxidizing agent [27–29]. Incubation of DNA with sulfite plus Cu^{2+} followed by the piperidine treatment gave a DNA cleavage pattern different from that induced by sulfite plus Co^{2+}.

The idea has been proposed that damage to DNA is a critical event not only in the initiation [30] but also in the promotion phase of carcinogenesis [31–33]. In the promion phase, the role of free radicals has been emphasized [31–33]. The possibility of participation of sulfur oxyradicals produced by sulfite plus metal ions in the promotion phase may be considered. Further research is necessary to clarify

whether sulfite plus metal-induced DNA damage occurs in the cells.

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